Impact of HIV infection in patients infected with chronic HCV (genotypes 1a and 3a): virological and clinical changes

Daryoush Behzadpour¹, Abbas Ahmadi Vasmehjani²,
Seyed Dawood Mousavi Nasab³, Nayeb Ali Ahmadi^{4,5}, Rasoul Baharlou²

¹Department of Virology, Rasht Branch, Islamic Azad University, Rasht, Iran, ²Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, ³Department of Research and Development, Pasteur Institute of Iran, Tehran, Iran, ⁴Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, ⁵Faculty of Paramedical Sciences, Department of Medical Lab Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection has become a serious public health problem. The influence of HIV/HCV coinfection on plasma HCV RNA loads and clinical criteria which are usually regarded as a predictor of the progress of liver disease have not been reliably evaluated.

Objectives: This study investigated the impact of HIV infection on HCV RNA load and clinical indexes in Yazd and Tehran.

Materials and methods: HCV/HIV-coinfected patients and HCV-monoinfected controls were examined and compared for plasma HCV RNA and related risk factors such as HCV genotypes, liver enzymes, and transmission routes.

Results: A total of 54 HCV/HIV-coinfected patients and 88 HCV-monoinfected controls were studied. The HCV RNA load mean was significantly higher in HCV/HIV-coinfected patients than in HCV-monoinfected patients ($p < 0.001$). HCV RNA load mean in patients infected with HCV without anti-HCV therapy was lower than HIV/HCV patients with and without highly active antiretroviral therapy that this difference was significant ($p < 0.001$). The HCV RNA levels were significantly higher in HIV/HCV genotype 3a coinfecting patients than in genotype 3a monoinfected patients ($p < 0.001$). HIV RNA levels were lower in genotype 1a infected patients than in genotype 3a infected patients, but this difference was not significant statistically. The ALT mean levels were significantly higher in genotype 3a HIV/HCV-coinfected patients than in genotype 3a HCV-monoinfected patients ($p < 0.001$).

Conclusions: HIV/HCV coinfection leads to a significant increase in plasma HCV RNA. Further evaluations of the effects of ART and HIV infection on the course of HCV infection and the response to treatment against HCV infection in other and different genotypes are also needed. Moreover, HIV-infected patients should be screened regularly for HCV coinfection, particularly if they are in high-risk groups such as IDUs and recipients of blood transfusions.

Keywords: Hepatitis C virus, HIV, Genotypes, Viral load, Coinfection

Introduction

Chronic hepatitis C virus (HCV)-infected individuals, 130–170 million of the world's population, are at high risk of progression to liver fibrosis and liver failures such as cirrhosis and end-stage liver disease, especially HCV/human immunodeficiency virus (HIV)-coinfected patients.¹ Likely because of possible transmission routes, HCV infection is highly prevalent (20–40%) in subjects

infected with the HIV.² In addition, this risk could be increased even more by coinfection with HIV or other viruses, which is likely to occur due to the transmission routes shared between HIV and HCV.^{3,4} For example, parenteral exposure modes such as intravenous drug users (IDUs) or multiple transfusions have been consistently afflicted and found to be the most important risk factors for coinfection of HIV with HCV.⁵ Interestingly, this rate of coinfection has varied significantly worldwide depending on facilitating risk factors such as IDUs, geographic regions, risk groups, and exposure type, which may be different in each region of the same country.^{6,7}

Correspondence to: Nayeb Ali Ahmadi. Proteomics Research Center, and Department of Medical Laboratory Technology, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Darband street, Tajrish, 1971653313 Tehran, Iran. Email: nayebalia@sbmu.ac.ir; nayebalia@yahoo.com

Different studies have indicated a high and changing rate of coinfection with HCV among human immunodeficiency virus 1 (HIV-1)-infected patients, especially among injecting drug users.^{8–11} Coinfection with HIV has a significant effect on the replication cycle of HCV and on the natural history of HCV infection and possibly vice versa.¹² However, coinfections of HCV and HIV have been associated with reduced survival; HIV coinfection considerably reduces the overall response rate to therapy against hepatitis C with pegylated interferon (Peg-IFN) plus ribavirin (RBV).^{13,14} The highly active antiretroviral therapy (HAART) program in HIV-infected patients has led to a reduction in HIV-related complications, and as a result, the impact of chronic HCV infection on morbidity and mortality in HIV-infected patients has become apparent.^{15,16}

HCV/HIV coinfection is correlated with progressive hepatic fibrosis and severe liver diseases, resulting in liver-related morbidity and mortality.¹⁷ Previous studies have indicated that HCV/HIV coinfection has a negative effect on CD4 cell population and even impacts adversely the clinical progression of HIV immunological and clinical chemistry observations.¹⁸ However, other reports have demonstrated that it may have an adverse effect despite a similar decrease in the HIV viral load in HIV-monoinfected and HIV/HCV-coinfected patients.^{19,20} It has also been reported that HCV RNA levels are higher in HIV/HCV-coinfected patients than in HCV-monoinfected subjects, which may be due to an inadequate immune control of HCV replication in HIV coinfection; the negative impact of HIV infection on anti-HCV treatment response has been demonstrated.²¹

HIV infection has been associated with higher rates of HCV persistence, which, it has been hypothesized, is related to its impact on cellular immune function. Studies of chronic HCV/HIV coinfection have revealed impaired HCV specific CD4 and CD8 T cell responses.²² Also, high HCV loads have been consistently associated with HIV coinfection. Associations with HCV genotype and degree of liver fibrosis, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and CD4 cell counts may correlate to high HCV RNA in plasma.²³ Furthermore, a positive correlation has been reported between viral load and some interleukins, and this is also affected by different HCV genotypes.^{24,25}

HIV infection has been reported as leading to a decrease in HCV clearance and increases in viral load and complications from the infection, but no consensus exists between these reports.²⁶ Reports also indicate that sustained response to anti-therapy against HCV among HCV/HIV-coinfected patients was decreased, but the precise underlying mechanism for this fact is not fully known.^{14,27} The likelihood of achieving a sustained virological response (SVR) is lower in HIV/HCV-coinfected persons than in those with HCV monoinfection, although this rate varies between genotypes. Hence, understanding the effect HIV infection has on HCV patients is essential in formulating any potential treatment plan. Therefore, the main objectives of this study were to investigate the influence of HIV

on plasma HCV RNA load in patients bearing HCV/HIV coinfection and to assess factors that influence HIV progression in respect to different HCV genotypes.

Materials and methods

Study design and population

In this study, the 142 participants were either infected with both HCV and HIV (i.e. HCV/HIV coinfection) or HCV-monoinfected and were registered at the Medical Clinic Centre in Yazd (central Iran) or in Tehran between September 2013 and February 2014.

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. Informed consent was obtained from each patient prior to participation. Diagnosis of chronic HCV infection is defined as positive anti-HCV antibodies or based on clinical and biochemical criteria (acute hepatitis in individuals without preexisting liver disease, excluding other infective and drug causes, and serum ALT levels higher than normal range) along with HCV RNA detectable by molecular methods. Diagnoses of HIV infection were based on serial ELISA and western blot tests. Selected study participants were arranged in groups as described in Table 1. The standard treatment protocol for chronic HCV infection is the combination of peginterferon alfa (PegIFN) plus ribavirin (RBV) for at least 24 and 48 weeks for 3a and 1a HCV genotypes, respectively. SVR is rather defined as an undetectable HCV RNA level (<50 IU/ml) 24 weeks after treatment end, and it indicates the good outcome of treatment. In HIV/HCV coinfection, patients used the minimal duration of anti-HCV treatment for 48 weeks. Also coinfection under HAART patients have been taking the same combination therapy (Zidovudine (ZDV) + Lamivudine (3TC) + Efavirenz (EFV)) and there was no considerable

Table 1 Clinical and laboratory parameters in HCV and HCV/HIV patients

Variables	HCV (n = 88)	HCV/HIV (n = 54)
Age	41.27 ± 11.18	39.46 ± 13.13
Sex		
Male	63 (71.6%)	43 (79.6%)
Female	25 (28.4%)	11 (20.4%)
ALT (IU/l) ^a	46.1 ± 17.11	55.14 ± 13.2
CD4 ⁺ T cell/μL		312.2 ± 134.1
<150	–	17 (31.5%)
≥150	–	37 (68.5%)
HCV load (IU/mL) ^a	697876 ± 48461	2415404 ± 318412
HIV load (IU/mL) ^a	–	1475481 ± 282382
Genotypes		
1a	33 (62.5%)	8 (14.8%)
3a	55 (37.5%)	46 (85.2%)
HCV treatment		
Yes	71 (80.7%)	9 (16.7%)
No	17 (19.3%)	45 (83.3%)
HIV treatment		
Yes	–	44 (81.5%)
No	–	10 (18.5%)
Infection transmission		
IUDs	71 (80.7%)	27 (50%)
Blood transfusion	4 (4.5%)	7 (13%)
Unknown	13 (14.8%)	20 (37%)

^aMean ± SEM.

difference on the duration of treatment of these patients, approximately three months.

Diagnostic tests

A third-generation ELISA (Dia. Pro, Italy) was used to determine anti-HCV. Positive results from both the western blot (for HIV) and third-generation ELISA (for HCV) were considered indicative of HIV/HCV coinfection. CD4⁺ cell counts were obtained through flow cytometry (Partec, Germany). The tests for biochemistry, liver function, and serum ALT levels were run using a commercially available Hitachi-7600 series analyzer.

HCV and HIV RNA quantification

Plasma HCV RNA (viral load) was extracted and HCV RNA levels were determined by qualitative reverse transcription-polymerase chain reaction (qRT-PCR) using TaqMan technology with commercially available kits (PCR-Fluorescent Probing, PG Biotech Ltd., Shenzhen, China). Probe signals were detected in real time (7500 Real-Time PCR system; Applied BioSystems, USA). Quantitative detection of plasma HIV RNA (viral load) was performed using a nucliSens easyQ HIV-1 assay (BioMérieux, Boxtel, the Netherlands). Typically, the lower limits of detection for HCV and HIV tests are 500 and 1000 IU/mL, respectively. HCV genotyping was performed using type-specific primers and the protocol described previously.²⁸

Statistical test

Statistical analysis was carried out using SPSS statistical software version 19.0. Variables were compared using the student's t-test. The chi-square test was applied to evaluate differences between categorical variables. Correlations between HCV RNA load and HIV RNA levels were evaluated by means of Spearman's test. A value of $p < 0.05$ was considered statistically significant for all tests.

Results

A total of 142 chronic HCV patients, 106 (74.6%) males and 36 (25.4%) females, were included in the study. Of

these individuals, 54 (38%) were HIV/HCV-coinfected. The mean age of the participants was 41.5. The main characteristics of the two groups are presented in Table 1.

The HCV RNA load mean was significantly lower in patients infected with and undergoing therapy against HCV than in untreated patients (998600 ± 1157000 copy/ml vs. 1806000 ± 2136000 , $p < 0.001$) that are shown in Fig. 1(a). The HIV RNA load mean was higher in patients infected with HCV and undergoing anti-HCV therapy than in untreated patients, but the difference was not significant (1644000 ± 2593000 vs. 1442000 ± 1989000 ,

Figure 1 Relation between the HCV RNA levels and HCV-infected patients with and without HIV RNA. (a) HCV RNA level in patients infected with HCV with and without therapy against HCV; (b) HCV RNA load in HCV/HIV-coinfected patients compared to monoinfected individuals. *** $p < 0.001$

Figure 2 HCV load level in HIV/HCV patients with and without therapy against HIV and HCV. (a) The comparison of HCV RNA level in untreated HCV with HCV/HIV coinfection with HAART, (b) HCV RNA level in treated HCV patients in HCV/HIV coinfection with HAART, (c) HCV RNA level in untreated HCV patients in HCV/HIV coinfection without HAART, (d) The HCV RNA level in HCV/HIV-coinfected patients underlying both HCV and HIV therapy. *** $p < 0.001$

$p > 0.05$). Interestingly, the HCV RNA load mean was significantly higher in HCV/HIV-coinfected patients than in HCV-monoinfected patients (2415000 ± 2340000 vs. 697900 ± 454600 , $p < 0.001$); the means are shown in Fig. 1(b).

HCV RNA load mean HCV patients with and without anti-HCV therapy was lower than HIV/HCV patients underlying HAART that this difference was significant ($p < 0.001$) Fig. 2(a) and (b). Also HCV RNA load mean in patients infected with HCV without anti-HCV therapy was lower than HIV/HCV patients without HAART that this difference was significant ($p < 0.001$) (Fig. 2(c)). The HCV RNA level in HCV/HIV-coinfected patients underlying both HCV and HIV therapy was significantly higher than HIV/HCV patients with and without anti-HCV and HAART ($p < 0.001$) (Fig. 2(d)).

Viral markers correlation in two patients groups based on different variables

HIV RNA levels in HIV/HCV-coinfected patients were lower in genotype 1a-infected patients than in genotype 3a-infected patients, but this difference was not statistically significant (662400 ± 501400 vs. 1617000 ± 2212000 , $p > 0.05$). The HCV RNA levels in HIV/HCV-coinfected and -monoinfected patients were determined in regard to two HCV genotypes. The HCV RNA load was significantly higher in genotype 3a-infected patients than in genotype 3a-monoinfected patients ($p < 0.001$); the results are shown in Fig. 3. A similar pattern was seen in the case of genotype 1a in HIV/HCV-coinfected and -monoinfected patients, but this difference was statistically significant ($p < 0.01$).

ALT mean levels in HIV/HCV-coinfected and -monoinfected patients were determined with regard to two HCV genotypes. The ALT mean levels were significantly higher in genotype 1a-infected patients related to HIV/HCV coinfection than in genotype 3a-infected patients ($p < 0.01$); that are shown in Fig. 4. Also, ALT mean levels were significantly higher in genotype 3a HIV/HCV-coinfected patients than in genotype 3a HCV-monoinfected patients ($p < 0.001$).

The HCV RNA levels between HIV/HCV-coinfected and -monoinfected patients based on infection transmission

Figure 3 The HCV RNA level in HIV/HCV-coinfected and -monoinfected patients with respect to two HCV genotypes. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$

Figure 4 The ALT mean levels in HIV/HCV-coinfected and -monoinfected patients with respect to two HCV genotypes. ** $p < 0.01$ and *** $p < 0.001$

Figure 5 The comparison of HCV RNA level between HIV/HCV-coinfected and -monoinfected patients based on infection transmission. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$

were compared. The results showed that HCV RNA levels in all manners of infection transmission were higher in HIV/HCV-coinfected patients than in monoinfected patients; however, levels were higher in recipients of blood transfusions than in patients coinfecting through other routes, and the difference was significant (Fig. 5).

Discussion

HIV infection could significantly affect the lifecycle of HCV and the immune system's ability to control HCV replication. As a result of its significant influence on the natural history of HCV infection, liver disease progression is accelerated and/or mortality rates are higher than in monoinfection patients. These results were consistently reported for multitransfused patients and from a similar survey of intravenous drug users (IDUs). These effects could be related to the immune system and/or a direct interaction between the viruses; therefore, immune suppression and the direct effect of HIV infection are not the only factors contributing to this condition. HIV coinfection has a significant early impact on the clinical, immunological, and virological outcomes of HCV infection in different stages of the disease. The current study demonstrates that HIV coinfection has a significant impact on the HCV RNA load that is associated with higher HCV viremia. This result is in accordance with previous studies.^{13,24} As has been noted in many studies, the current study also

found significantly more high HCV RNA loads in patients with HIV/HCV coinfection than in HCV monoinfected patients.^{29,30} However, in another study, HCV-monoinfected patients were more likely to have higher HCV RNA loads; because of this contrast, coinfecting patients are defined by a reduced possibility of HCV clearance and higher levels of HCV RNA.^{31–33} Therefore, HIV coinfection as an independent factor can threaten HCV patients. It is noteworthy that treatment of HCV was seen not to affect HIV RNA levels. This has also been seen in other reports, mostly of HIV/HCV-coinfecting patients treated against HCV receiving effective antiretroviral therapy (ART) (reduced HIV RNA level and higher CD4 cell counts). This finding may support the hypothesis that it is unlikely that a poorer response to FDA-approved therapy against HCV will be observed in HIV/HCV-coinfecting individuals as compared to the HCV-monoinfected population. Furthermore, the response rates are still lower in these patients than in those without HIV infection, which may be attributed to HCV RNA levels. Although HIV infection alone seems to have no significant impact on HCV viral replication, ART had a positive impact on HCV viral replication or a negative impact on HCV viral suppression among HCV/HIV-coinfecting patients.^{34,35} Consequently, the results of this study may suggest a possible antiviral effect against HCV in contrasting HIV RNA levels on controlling the replication of HIV in parallel to the effect of antiretroviral therapy against the life cycle of HCV. This may be better demonstrated in a larger population.

One of the major predictive factors for SVRs is HCV genotype; therefore, because of the effect HIV/HCV coinfection, the HCV genotype was evaluated. Interestingly, different HCV genotypes could have varying effects on liver cells; nevertheless, the importance of these genotypes in HCV/HIV coinfection is critical. In addition, HCV clearance is correlated with a strong CD4⁺ T helper cell response. HIV/HCV coinfection may be correlated with the accelerated decline in these patients occurring in part because they have diminished cellular immune responses to HCV infection and weak CD8⁺ T cell and CD4⁺ T-cell immune activity.^{26,36} Of course, ART leads to an increased CD4⁺ T cell level and enhanced immune response resulting in the destruction of infected hepatocytes and an increased HCV viral load. These conditions may vary in different HCV genotypes.^{35,37} The present study indicated that HCV RNA levels were significantly higher in HCV/HIV-coinfecting patients contaminated with genotypes 1a and 3a than in monoinfected patients; this difference between the two genotypes in HCV/HIV patients was not seen. This finding may indicate that the influence of HIV infection on HCV RNA levels between HCV genotypes 1a and 3a in coinfecting patients are not varied; rather, those in monoinfected patients are higher. However, this finding may result from the effect of HCV and HIV treatment, because SVR rates in different HCV genotypes were varied.

Significantly higher ALT levels were found in HIV/HCV-coinfecting patients than in HCV-monoinfected patients. Interestingly, this level was higher in genotype 3a-coinfecting patients than in other groups. Also as noted, higher ALT levels were independently associated with faster fibrosis progression, and, in HIV/HCV-coinfecting patients, a CD4 count <250 is independently associated with advanced liver disease. Therefore, ALT levels could vary among different genotypes. Several studies demonstrated that in HCV/HIV-coinfecting patients, the degree of inflammation in the liver and the rate of fibrosis progression are higher than those in HCV-monoinfected patients.¹⁸

Intravenous drug use is the predominant mode of transmission of HCV in the world.^{4,38} Approximately 75% of HIV-positive people in Iran are IDUs.³⁹ The present study indicated that blood transfusion was the predominant route of HIV/HCV-coinfecting transmission than monoinfected patients. Of course, HCV RNA levels in all transmission routes studied in this research in HIV/HCV-coinfecting patients were significant higher than in HCV-monoinfected patients; this result may support the influence of HIV infection on HCV RNA levels with respect to transmission routes, especially through blood transfusion.

In conclusion, HIV/HCV coinfection leads to a significant increase in plasma HCV RNA. Therefore, it is unlikely that differences in HCV RNA load may contribute significantly to the poorer response rates to anti-HCV therapy hypothesized in HIV/HCV-coinfecting patients. Further evaluation of the effect of ART and HIV infection on the course of HCV infection and the response to treatment against HCV infection in other and different genotypes are also needed. HIV-infected patients should be screened regularly for HCV coinfection, particularly if they are in high-risk groups such as IDUs and recipients of blood transfusions.

Conflict of interest

The authors declare that there is no conflict of interests.

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